Nitrate Stress Response in *Desulfovibrio vulgaris* Hildenborough: Whole-Genome Transcriptomics and Proteomics Analyses

Q. He[1,2], Z. He[4,2], W. Chen[3], Z. K. Yang[2], E. J. Alm[5], K. H. Huang[5], H-C. Yen[6], M. Keller[3], T. C. Hazen[5], A. P. Arkin[5,7,8], J. D. Wall[6], J. Zhou[2,4]

VIMSS Virtual Institute for Microbial Stress and Survival

http://vimss.lbl.gov/

[1]Temple University, Philadelphia, PA.
[2]Oak Ridge National Laboratory, Oak Ridge, TN.
[3]Diversa Corp., San Diego, CA.
[4]University of Oklahoma, Norman, OK.
[5]Lawrence Berkeley National Laboratory, Berkeley, CA.
[6]University of Missouri, Columbia, MO.
[7]University of California, Berkeley, CA.
[8]Howard Hughes Medical Institute, Chevy Chase, MD.

Abstract

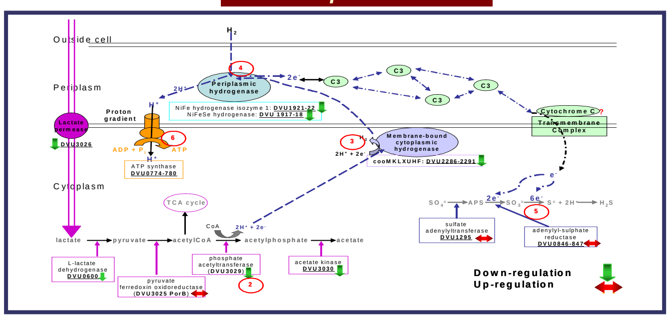
Sulfate reducing bacteria (SRB) are of interest for bioremediation with their ability to reduce and immobilize heavy metals. Nitrate, a common co-contaminant in DOE sites, is suggested to inhibit SRB via nitrite. Previous results indicate that nitrite is indeed inhibitory to the growth of *Desulfovibrio vulgaris*. However, growth inhibition by nitrate alone was also observed. In this study, growth and expression responses to various concentrations of nitrate were investigated using the Omnilog phenotype arrays and whole-genome DNA microarrays. Changes in the proteome were examined with 3D-LC followed by MS-MS analysis. Microarray analysis found 5, 50, 115, and 149 genes significantly up-regulated and 36, 113, 205, and 149 down-regulated at 30, 60, 120, and 240 min, respectively. Many of these genes (~50% at certain time points) were of unknown functions. By comparison to NaCl stress, transcriptional analysis identified changes specific to $NaNO_3$ stress. The hybrid cluster protein was among the highly up-regulated genes, suggesting its role in nitrate stress resistance with its proposed function in nitrogen metabolism. The up-regulation of phage shock protein genes (*pspA* and *pspC*) might indicate a reduced proton motive force and the repression of multiple ribosomal protein genes could further explain the growth cessation resulting from nitrate stress. A glycine/betaine transporter gene was also up-regulated, suggesting that NaNO3 also constituted osmotic stress. Osmoprotectant accumulation as the major resistance mechanism was validated by the partial relief of growth inhibition by glycine betaine. Proteomics analyses further confirmed the altered expression of these genes, and in addition, detected increased levels of several enzymes (Sat, DvsB, and AprB) in the sulfate reduction pathway, indicative of the increased energy production during nitrate stress. In conclusion, excess NaNO3 resulted in both osmotic stress and nitrate stress. *D. vulgaris* shifted nitrogen metabolism and energy production in response to nitrate stress. Resistance to osmotic stress was achieved primarily by the transport of osmoprotectant.

Materials and Methods

Oligo Probe Design and Microarray Construction: 70mer oligo probes were designed using a computer software ArrayOligoSelector (Zhu 2002) combined with other programs such as Oligopicker (Wang and Seed 2003) and OligoArray (Rouillard et al., 2002). The whole genome microarray covers 3574 ORFs, including 3471 (97.1%) unique probes and 103 (2.9%) probes which may cross-hybridize with other sequences. Oligo probes were spotted onto UltraGAPSTM coated glass slides (Corning Life Science, NY) using a Microgrid II robotic arrayer (Genomic Solutions Inc., MI).

3D-LC-MS/MS Proteomics Analysis: Fractionation of total proteins by three-dimensional LC followed by MS/MS analysis for protein identification. Relative abundance of proteins was estimated by the total numbers of qualified spectral counts representative of the relative abundance of each protein. Proteins that have significant changes were identified using the statistical "local-pooled-error" test by selecting changers with $p < 0.05$.

Conceptual Model



Acknowledgements

This research was funded by the U.S. Department of Energy (Office of Biological and Environmental Research, Office of Science) grants from the Genomes To Life Program. Oak Ridge National Laboratory is managed by University of Tennessee-Battelle LLC for the Department of Energy under contract DOE-AC05-00OR22725.

DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT
OFFICE OF SCIENCE U.S. DEPARTMENT OF ENERGY

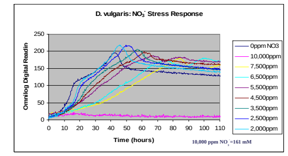
Results

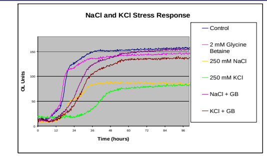
Proteins with Significantly Higher Abundance under Nitrate

GeneID	Symbol	Descriptions	p-value
DVU0072	-	glucose-1-phosphate cytidylyl-transferase	0.018
DVU0261	-	universal stress protein family	0.034
DVU0326	hypE	hydrogenase expression/formation protein HypE	0.000
DVU0386	-	"amino acid ABC transporter, periplasmic amino acid-binding protein"	0.000
DVU0403	dvsB	dissimilatory sulfite reductase beta subunit	0.009
DVU0414	-	NADP-dependent malic enzyme-related protein	0.008
DVU0441	ade	adenine deaminase	0.041
DVU0470	trpB-2	"tryptophan synthase, beta subunit"	0.046
DVU0547	-	"high-affinity branched chain amino acid ABC transporter, periplasmic	0.000
DVU0795	purC	phosphoribosylaminoimidazole-succinocarboxamide synthase	0.014
DVU0846	aprB	"adenylylsulphate reductase, beta subunit"	0.032
DVU1025	upp	uracil phosphoribosyltransferase	0.029
DVU1095	argG	argininosuccinate synthase	0.041
DVU1179	aor	"aldehyde:ferredoxin oxidoreductase, tungsten-containing"	0.019
DVU1282	glmM	phosphoglucosamine mutase	0.002
DVU1295	sat	sulfate adenylyltransferase	0.040
DVU1424	gcvPB	"glycine cleavage system P protein, subunit 2"	0.033
DVU1470	ppiC	peptidyl-prolyl cis-trans isomerase C	0.021
DVU1593	cheY-1	chemotaxis protein CheY	0.016
DVU1868	dapA	dihydrodipicolinate synthase	0.028
DVU2013	-	hybrid cluster protein	0.007
DVU2286	-	"hydrogenase, CooM subunit, putative"	0.036
DVU2290	-	"hydrogenase, CooU subunit, putative"	0.006
DVU2297	-	"glycine/betaine/L-proline ABC transporter, periplasmic-binding protein	0.011
DVU2885	-	"alcohol dehydrogenase, iron-containing"	0.034
DVU3094	rr	rubrerythrin	0.004
DVU3176	-	UDP-glucose/GDP-mannose dehydrogenase family protein	0.016
DVU3184	-	rubredoxin	0.000
DVU3210	thrC	threonine synthase	0.007
DVU3228	cheY-3	chemotaxis protein CheY	0.044
DVU3294	-	aldehyde dehydrogenase (NADP) family protein	0.019
DVU3371	metE	5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransfera	0.031

Proteins with Significantly Lower Abundance under Nitrate

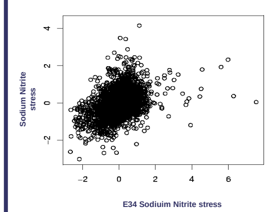
GeneID	Symbol	Descriptions	p-value
DVU0503	pnp	polyribonucleotide nucleotidyltransferase	0.000
DVU0777	atpA	"ATP synthase, F1 alpha subunit"	0.029
DVU0987	-	heavy metal-binding domain protein	0.000
DVU1303	rplC	ribosomal protein L3	0.004
DVU1306	rplB	ribosomal protein L2	0.004
DVU1308	rplV	ribosomal protein L22	0.007
DVU1317	rpsH	ribosomal protein S8	0.007
DVU1321	rpmD	ribosomal protein L30	0.046
DVU1326	rpsM	ribosomal protein S13	0.000
DVU1327	rpsK	ribosomal protein S11	0.000
DVU1330	rplQ	ribosomal protein L17	0.018
DVU1443	flgE	flagellar hook protein FlgE	0.000
DVU1545	-	hemolysin-type calcium-binding repeat/calx-beta domain protein	0.012
DVU1896	rpsT	ribosomal protein S20	0.000
DVU2325	merP	mercuric transport protein periplasmic component	0.001
DVU2364	-	"aminotransferase, classes I and II"	0.024
DVU2481	-	"formate dehydrogenase, beta subunit, putative"	0.002
DVU2519	rpsI	ribosomal protein S9	0.049

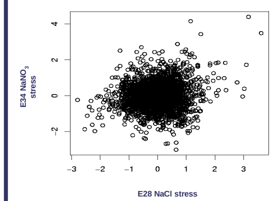


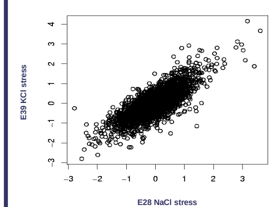


➢ Compared with NaCl/KCl, *D. vulgaris* is more sensitive to $NaNO_3$.

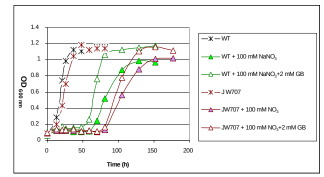
Comparison of Transcriptional Responses

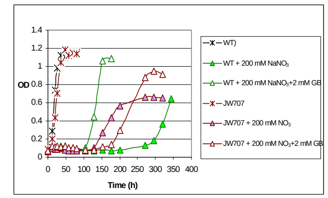






Effect of Compatible Solutes





➢ Glycine betaine (GB) relieved nitrate stress in *D. vulgaris*, consistent with the up-regulation of GB transporter gene.

Up-regulation genes:
- ➢ Hybrid cluster protein gene
- ➢ Phage shock protein genes
- ➢ Glycine betaine transporter genes
- ➢ Genes in the sulfate reduction pathway

Down-regulation genes:
- ➢ Multiple genes encoding ribosomal proteins

- *D. vulgaris* is more sensitive to $NaNO_3$ Stress than to NaCl/KCl stress.
- Very different responses at transcriptional level between $NaNO_3$ Stress and NaCl stress.
- Indicative of stress response pathways other than those of salt stress.

Summary

1. Excess $NaNO_3$ resulted in both osmotic stress and nitrate stress.
2. Hybrid cluster protein gene was highly up-regulated, suggesting N metabolism involved in nitrate resistance.
3. Up-regulation of phage shock protein genes indicated energy deficiency resulting from nitrate stress
4. Sulfate reduction pathway up-regulated to generate more energy.
5. Repression of multiple ribosomal protein genes could explain the growth cessation.
6. Glycine/betaine transporter genes were up-regulated, indicating that $NaNO_3$ also constituted osmotic stress.
7. Resistance to osmotic stress was achieved primarily by osmoprotectant accumulation.